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# INCREASED ANALGESIA TO THERMAL STIMULI IN RATS AFTER BRIEF EXPOSURES TO COMPLEX PULSED 1 MICROTESLA MAGNETIC FIELDS[1]

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*Summary.*—Nociceptive thresholds to a 55°C hot surface were measured for female Wistar rats before treatments and 30 min. and 60 min. after the treatments. After injection with either naloxone or saline following baseline measurements, the rats were exposed for 30 min. to either sham fields or to weak (about 1 microTesla) burst-firing magnetic fields composed of 230 points (4 msec. per point) presented once every 3 sec. The rats that had received the burst-firing magnetic fields exhibited elevated nociceptive thresholds that explained about 50% of the variance. A second pattern, designed after the behaviour of individual thalamic neurons during nociceptive input and called the "activity rhythm magnetic field" produced only a transient analgesic effect. These results replicated previous studies and suggest that weak, extremely low frequency, pulsed magnetic fields with biorelevant temporal structures may have utility as adjuncts for treatment of pain.

We have been testing the validity of the assumption that the specificity of intervention afforded by a pharmacological agent's spatial (molecular) structure can be duplicated by a weak, pulsed magnetic field's temporal pattern. Fleming, Persinger, and Koren (1994) had showed that exposure to a computer-generated 1 microTesla to 5 microTesla (10 to 50 milligauss) burst-firing magnetic field (230 points, 3 msec. per point) once every 4 sec. for 20 min. elevated the nociceptive threshold to footshock in rats. The strength of the analgesic effect 40 min. later (20 min. following the exposure) was more than the residual analgesia induced by 4 mg/kg of morphine. Previous studies by Thomas, Kavaliers, Prato, and Ossenkopp (1997) found that the frequency-modulated patterns of magnetic fields with intensities within the range of 100 microTesla also induced antinociception (analgesia) in land snails.

However, when rats were exposed to a frequency-modulated (Thomas) pulse whose point duration was 1 msec. compared to 3 msec. every 4 sec. for 30 min., no analgesic response to thermal stimuli was observed. Instead, exposure to this pattern abolished the analgesic effects induced by morphine or L-NAME (L-Nitro-Arginine Methyl Ester), an inhibitor of nitric oxide

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synthase, when they were injected separately but not when injected together (Dixon & Persinger, 2001). We designed the present study to discern if exposure of rats to the original burst-firing pattern (Fleming, *et al.*, 1994) would produce analgesia in the thermal nociceptive paradigm (hot-plate response latency) comparable to that observed in the paradigm of flinch threshold to electric shock.

## Method

### *Subjects*

In two experiments, 36 female albino Wistar rats, approximately 100 to 150 days of age, were employed as subjects. They had been maintained three per cage in standard wire cages within controlled colony conditions. The light:dark cycle was 12:12, and temperature varied within 1° of 20°C.

### *Procedure*

After approval by the Animal Care Committee, rats for both experiments were removed from their home cages and brought to an experimental room where the nociceptive thresholds were obtained. Each rat was placed on a hot-plate designed for this task that was maintained at 55°C. The time in seconds between the placement of the rat on the plate and two licks of either hind paw by the rat was recorded manually. The rat was immediately removed when this criterion was achieved or when 60 sec. had elapsed. The rats were then administered either the saline or the naloxone and exposed to either the magnetic field or the sham field and returned for a second measurement 30 min. later. After the second measurement the rats were returned to their home cages until the third test was initiated 30 min. later. This procedure was repeated for two successive days.

In Exp. I, after the baseline measurement was obtained, 10 rats received single intraperitoneal injections of naloxone (10 mg/kg) while another 10 rats received single intraperitoneal injections of isotonic saline. Half the numbers of rats in each group were exposed to either the burst-firing magnetic field or to the sham-field condition. Pairs of rats were placed in a 30-cm × 30-cm × 30-cm plastic cage. Two solenoids, created by wrapping of wire around large nails (Fleming, *et al.*, 1994) were connected in series such that a field was generated across the cage. The plane of the horizontal placement of the two solenoids was equivalent to the height of a rat with all four legs on the 3 cm of corncob bedding.

During the field condition, the rats were exposed to 230 point values between 0 and 256 whose order and correlative voltage produced a burst-firing magnetic field. The duration of the point value was 4 msec., and the complete pattern was presented once every 3000 msec. (3 sec.) for 30 min. The pattern was generated by an IBM XT personal computer whose output

was delivered to a dc to ac converter before the current was applied through the solenoids. The magnetic field strength within the cage varied from 10 milliGauss (1 microTesla) in the middle to 25 mG (2.5 microTesla) nearest the solenoids on each side of the cage. During the sham-field condition, the equipment was not activated. The background strength of 60 Hz fields was less than 0.2 mG.

In Exp. 2, eight rats were exposed to the sham-field condition while another eight rats were exposed to the "activity rhythm pattern that was extracted from graphs of the activity of individual neurons within the nucleus ventralis posterior of the thalamus after a single electric pulse was applied to the sciatic nerve of the left leg of a rat (Emmers, 1981). This predictable pattern had been called the Activity Rhythm pulse by Emmers. In this experiment there were 140 points. The duration of each point was 2 msec. The time between the first two pulses (2 msec. on, 2 msec. off, 2 msec. on) and the three wider pulses (duration = 38 msec.) was 160 msec., so the total duration was 280 msec. This pattern was presented once every 440 msec. to simulate the firing pattern of large populations of thalamic neurons. The intensities of the magnetic fields within the exposure area were within the same range as those generated in Exp. I.

The absolute times required to lift (lick) one of the hind paws at baseline, +30 min. and +60 min. for the two successive days were analyzed. Additional measures, to minimize individual differences, involved (1) $z$ scores calculated by subtracting each score for each rat from the mean of its six scores and dividing by the standard deviation of those scores and (2) calculation of the relative change on each day by dividing the latencies at +30 and +60 min. after treatment by the baseline for that day. The general analyses were mixed within- and between-subjects' designs. *Post hoc* analyses involved Tukey's and paired $t$ tests. All analyses employed SPSS software on a VAX computer.

## Results

Four-way analysis of variance for the time required to lift the hind feet demonstrated no statistically significant differences between rats' performances across magnetic field treatments ($F_{1,17} = 1.45$, $p > .05$) or drug treatment ($F_{1,17} < 1.00$). The interaction was also not significant ($F_{1,17} < 1.00$). However, the interactions between trials and presence or absence of the field ($F_{2,34} = 5.86$, $p < .01$; $\eta^2 = 25\%$) and trials and the injection of naloxone ($F_{2,34} = 4.60$, $p < .01$; $\eta^2 = 21\%$) were statistically significant. Except for the interaction between field treatment and day ($F_{1,17} = 7.23$, $p = .01$) and the interaction between days and trials ($F_{2,34} = 7.57$), no other effects were statistically significant.

The major source of the interaction between field and trial was due to

the greater response latencies in the rats that had been exposed to the burst-firing magnetic fields for 30 min., these latencies still being present compared to the sham-field controls 30 min. after the animals had been removed from the field. The means and standard deviations of these values for the baseline, +30-min., and +60-min. data were 16.5 ($SD$=5.4) sec., 15.2 ($SD$= 3.1) sec., and 14.7 ($SD$=3.1) sec. for the sham-field rats and 15.7 ($SD$= 3.8) sec., 18.9 ($SD$=4.9) sec., and 17.9 ($SD$=4.3) sec. for the field-treated rats. *Post hoc* Tukey analysis ($p<.05$) indicated that the source of the interaction between trials and drug treatment was due to the shorter latencies in responding to the hot plate 30 min. after the injection of naloxone as compared to response latencies in rats that had received saline.

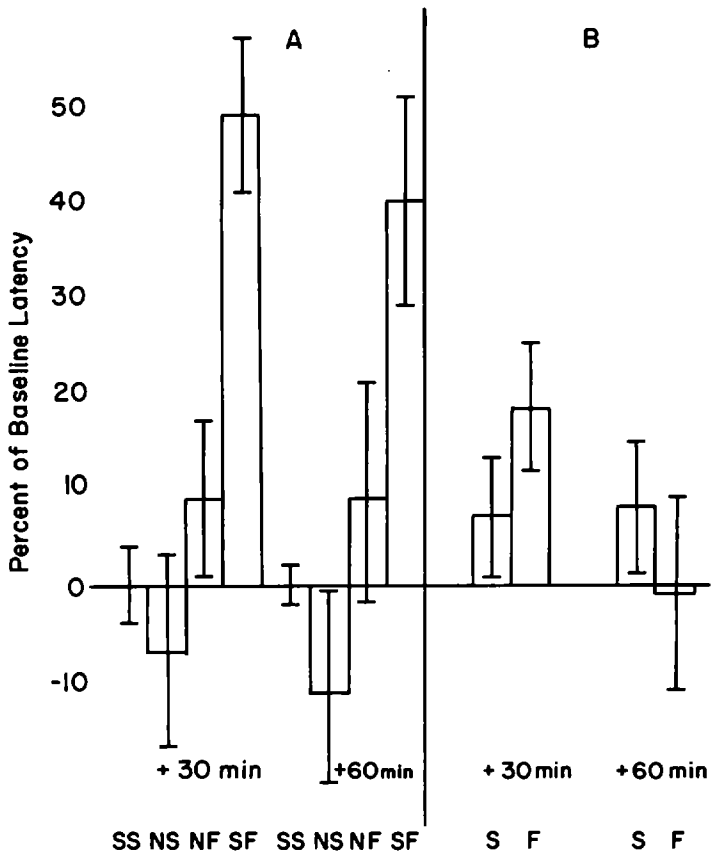


FIG. 1. Percent increase in latency to lift the hind paw relative to baseline, 30 min., and 60 min. (30 min. after no field) after initiation of treatment. (A) Burst-firing field: SS = saline-sham; NS = naloxone-sham, NF = naloxone-field, SF = saline-field. (B) Thalamic neuron field: S = sham, F = field.

The results of the analyses of the $z$ scores, where each rat's responses were norm-referenced against its own performance, verified the statistically significant interaction between exposure to the field and trial ($F_{2,34} = 6.69$, $p < .001$; $\eta^2 = 28\%$) but not the interaction between trial and drug ($F = 1.99$). A four-way analysis of variance for the relative change in latency 30 min. after exposure and 60 min. after exposure (30 min. of no exposure) showed a statistically significant elevation in relative response latency ($F_{1,17} = 18.99$, $p < .001$; $\eta^2 = 53\%$) after the field exposure when compared to the sham-field exposure but a significant reduction in relative response latency ($F_{1,17} = 8.99$, $p < .01$; $\eta^2 = 35\%$) after the injection with naloxone. The interaction between naloxone and field treatments fell short of statistical significance ($F_{1,17} = 3.44$, $p = .08$; $\eta^2 = 17\%$). The means and standard errors of the mean for the relative values for the four groups are shown in Fig. 1A.

Three-way analysis of variance as a function of trials, days, and exposure to the activity rhythm magnetic field or sham field showed no statistically significant effects for treatment ($F_{1,14} = .05$, $p > .05$) and no interactions between treatment and day ($F_{1,14} = .01$, $p > .05$), treatment and trial ($F_{2,28} = 1.48$, $p > .05$), or treatment, trial, and day ($F_{2,28} = .90$, $p > .05$). However, for the relative response latencies there was a statistically significant interaction ($F_{1,14} = 7.38$, $p < .01$; partial $\eta^2 = 35\%$) between treatment and trial. *Post hoc* analyses indicated that the major source of the interaction was the significant decrease in response latency 30 min. after the removal from the activity rhythm field compared to immediately after the 30-min. exposure, whereas these changes did not occur for the sham-field rats. The means and standard errors of the mean for these values are shown in Fig. 1B.

## Discussion

The results of this study are consistent with the observations by Fleming, *et al.* (1994). The novel contribution of the present study was the use of heat as a nociceptive stimulus rather than electric current. The main difference between these two methods of nociceptive testing is that electric shock is a polymodal stimulus that results in synchronous activation of all primary afferent neurons, while the thermal exteroceptive stimulus activates only those peripheral neurons that selectively respond to thermal nociception. The effect sizes of the magnetic field treatments and the persistence of analgesia for an additional 30 min. after the removal from the magnetic fields were comparable in both studies wherein the points' durations were 4 msec. and the time between the presentations of patterns was 3 sec. We had selected this interval initially because the work by Lewis, Cannon, and Libeskind (1980) had shown that application of direct nociceptive stimuli by electric current to the foot pads once every 4 sec. resulted in the induction of an endogenous opioid-mediated analgesia in rodents that could be blocked by naloxone, a potent, competitive, nonselective opiate receptor antagonist.

Although the interstimulus interval is an important factor, the shape of the complex magnetic field is also important. Dixon and Persinger (2001) exposed rats to the same hotplate procedure employed in this study and found that a frequency-modulated (Thomas) pulse did not produce analgesia even though the interstimulus interval was 4 sec. In this instance the point duration was 1 msec. In light of the greater efficacy of 3-msec. point durations, as compared to 1-msec. point durations to evoke electrical lability in limbic epileptic rats exposed to the same frequency-modulated pulse (Persinger & Belanger-Chellew, 1999), the confounding variable in this instance may likely have been the duration of the values that composed the pattern.

The results in Fig. 1A suggest that naloxone reduced the analgesic effects of the pulsed magnetic field; however, the interaction between the drug and field application fell short of statistical significance. That the dosage employed, i.e., 10 mg/kg, was sufficient to produce hyperalgesia was indicated by the significant decrease in response latency for rats which had received this treatment over responses by those which had not. The generalizability of the analgesic effect of pulsed magnetic fields to other species, such as snails (Thomas, *et al.*, 1997), suggests that a fundamental electromagnetic algorithm for this elementary and important sensory modality may be present across the phylogenetic spectrum.

The much weaker analgesic effect obtained from the magnetic field pattern that was tailored after the activity of single thalamic neurons responding to nociceptive stimuli was also transient. Unlike the effects of the burst-firing magnetic field presented once every 3 sec., the analgesic effects generated by the activity rhythm pulse were not present 30 min. later. If nociception is frequency-coded within the thalamic neurons or determined by the temporal pattern of neuronal firing as predicted by Emmers (1981), then our applied activity rhythm magnetic field may have mildly attenuated the neuronal signature within thalamic neurons associated with thermal nociception to the footpads.

Other research indicates that as the temporal structure of the applied magnetic field approaches the natural, neurogenic pattern, interference becomes more likely (McKay, Persinger, & Koren, 2000). We hypothesize that the mild analgesic effect from the activity rhythm magnetic field may not be mediated by opiate-related biochemistry but directly influence the firing patterns of thalamic neurons. Like the hypothesized virtual ligand, the effect dissipated when the complex magnetic field was removed.

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